Oxidation of humic substances by manganese oxides yields low-molecular-weight organic substrates

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MANY bacteria oxidize thermodynamically unstable manganese(II) to Mn oxides and deposit the oxides on their surfaces[1,2], a process that appears to account for most Mn oxidation in natural waters[3–5] and sediments[6]. Among the reasons that have been proposed for the evolutionary selection of this process are protection from damage by toxic metals and oxygen species, protection from ultraviolet light, and strengthening of the bacterial sheath or capsule[1,7]. Mn oxides may promote harmful free radical reactions, however, and marine Mn-oxidizing bacteria are particularly susceptible to photoinhibition[8]. Here we report that Mn oxides lyse complex humic substances, which in general cannot be used by microorganisms directly[9–11], to form low-molecular-weight organic compounds that can be used as substrates for microbial growth. Mn-oxidizing bacteria may thus be able to use the carbon pool in humic substances, which represent one of the largest organic reservoirs in natural waters, sediments and soils.

Mn oxides are reductively dissolved by humic and fulvic acids[12,13] and by certain simple organic compounds such as phenols and organic acids (such as pyruvate and oxalate)[14,15]. The products from the oxidation of humic substances by Mn oxides have not been investigated, although it is likely that a variety of low-molecular-weight (LMW) carbon compounds will be produced (in addition to CO_2 and CO) because of the heterogeneous nature of these substances. To test this hypothesis, we added synthesized Mn oxides to filtered estuarine water containing a high concentration (4.1 mg l^{-1}) of natural organic carbon and to low organic sea water from the Gulf of Mexico amended with different additions of isolated fulvic acids. Following addition of the oxides, the suspensions were filtered through 0.2 μm-pore nylon filters and the concentrations of LMW aldehydes and ketones[16] and α-ketoacids[17] were measured in the filtrates by high-performance liquid chromatography (HPLC).

A variety of LMW carbonyls were produced from the oxidation of dissolved natural organic matter (NOM) by the Mn oxides, including pyruvate, acetone, formaldehyde, acetaldehyde (in descending order of importance), and an unkown (Fig. 1). In experiments with fulvic acids (Fig. 2), the initial rates of production of pyruvate and acetaldehyde conformed to a Langmuir adsorption isotherm:

$$\mathrm{d}P/\mathrm{d}t = \frac{V_{\max}[\mathrm{FA}]K}{[\mathrm{FA}]K+1} \qquad (1)$$

where $\mathrm{d}P/\mathrm{d}t$ is the formation rate of product, [FA] is the concentration of fulvic acids, K is a pseudoequilibrium adsorption constant, and $V_{\max}$ is the maximum reaction rate achieved when all adsorption sites are saturated. A similar hyperbolic rate law has been found to describe the rate of reduction of Mn oxides by fulvic acids[13]. The fit of the reaction rate to an adsorption isotherm supports the general model of rapid adsorption of organic molecules onto Mn oxides followed by slower electron transfer at the oxide surface to produce Mn(II) and organic oxidation produces[18].

Nonlinear least-square fits of our fulvic acid rate data to equation (1) yielded $V_{\max}$ values of 36±2 (±s.d.) and 2.4±0.1 nM h^{-1} and values for K of 0.18±0.03 and 0.13±0.02 l mg^{-1} (at pH 8.0) for pyruvate and acetaldehyde, respectively (Fig. 2). These K values are in the same range as those (0.16 at pH 4 and 0.023 l mg^{-1} at pH 7.1) determined in ref. 13 for the rate of reduction of Mn oxides by the fulvic acids in 0.05 M NaCl. Comparisons between our results and those from ref. 13 are particularly appropriate because both studies used oxides synthesized by similar methods, and both used samples of the same Suwanee River fulvic acid preparation. A similarity in K values would be expected because both Mn(II) and the two LMW carbonyls are produced from the same reaction: oxidation of adsorbed fulvics by the Mn oxides. Our slightly higher K values (at pH 7–8) may reflect increased fulvic acid adsorption onto Mn oxides with increased salinity as a result of electrostatic screening of negative charge.

A time-course experiment in filtered water from the mouth of the Shark River estuary, Florida, showed a rapid production of formaldehyde and acetaldephyde during the first few hours after oxide addition followed by decreasing rates thereafter and little production after 21 h (Fig. 3). In a second experiment in this water, the net soluble yields of these two compounds after 14 h increased with oxide concentration, and were linearly related ($r^2=0.995$ and 0.993) to the amount of NOM removed due to reaction with the oxides as quantified by the decrease in NOM absorbance in sample filtrates at 300 nm (Fig. 4). Yields for pyruvate and acetone showed considerably different behaviour; their net production reached maxima and then decreased with increasing oxide concentrations (Fig. 4). These data are consist-

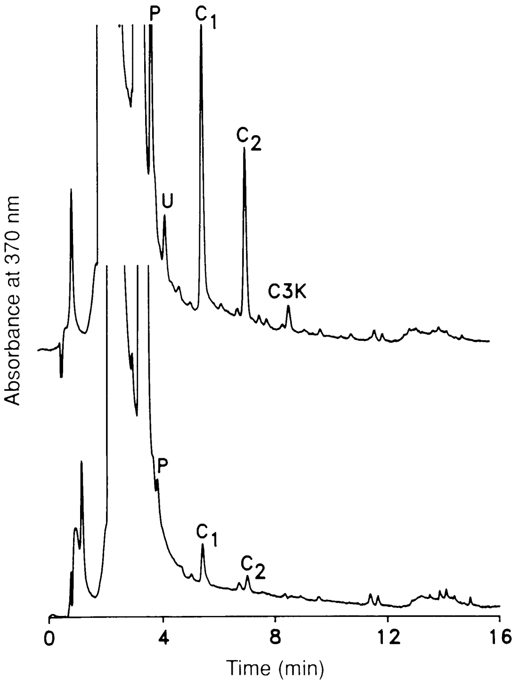

FIG. 1 HPLC chromatograms for dissolved LMW carbonyls in estuarine water initially (lower) and after 14.3 h exposure to 1 mM Mn oxides at pH 7.7 (upper). The peaks indicated are for the hydrazone derivatives[17] of pyruvate (P), formaldehyde (C_1), acetaldehyde (C_2), acetone (C3K), and one unknown (U). Chromatograms used to quantify pyruvate (using a mobile phase of higher pH[19]) are not shown. The 22‰ salinity water was collected from the estuary of the Shark River, which drains in part the Everglades Swamp in southernwestern Florida. It was stored in a high density polyethylene carboy in the dark at 4 °C for 10 months and was filtered through a 0.2 μm pore Nylon filter just before use. Manganese oxides were prepared from alkaline permanganate oxidation of $MnCl_2$ (ref. 21), a procedure that yields oxides of Mn oxidation state 3.8–4.0 (refs 13, 21). All experiments were done at 23±1 °C.

ent with subsequent oxidation of pyruvate[15] and actone on the oxide surface following their production. The linear yield behaviour of formaldehyde and acetaldehyde may be related to their greater resistance toward oxidation and/or to a greater tendency of these C_1 and C_2 compounds to desorb from the oxide following their formation.

Results of the above experiment were used to estimate yields for carbonyl products as a percentage of natural organic carbon (NOC). Yields were computed from initial slopes of relationships between molar concentration of product and delta absorbance (Fig. 4) multiplied by the number of carbon atoms per compound. The resulting values were divided by the ratio of NOC to absorbance for the estuarine water (2.2 mmol C l^{-1}/absorbance at 300 nm). In these computations we assumed that the NOC adsorbed and/or reacted is proportional to the absorbance lost from solution, a reasonable assumption given the linear relationship between concentrations of C1 and C2 products and delta absorbance. We estimate that 0.32, 0.128, 0.0079 and 0.0067% of the organic carbon reacted was converted to pyruv-

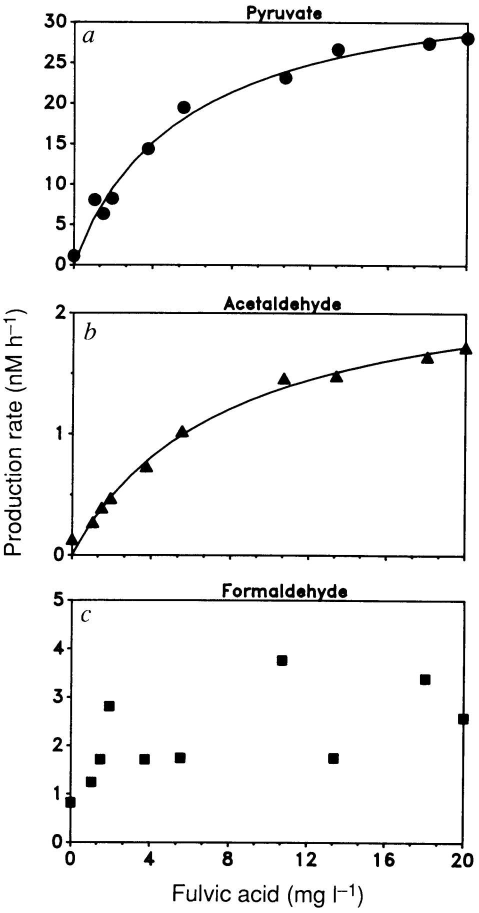

FIG. 2 Initial formation rates of *a*, pyruvate, *b*, acetaldehyde, and *c*, formaldehyde at pH 8.0 ± 0.1 in Gulf of Mexico water containing 1 mM oxides and 0–20 mg l^{-1} fulvic acids isolated from the Suwanee River, Georgia[19]. Rates were determined by measuring filterable carbonyl concentrations immediately after oxide addition and after 1.5 h reaction. They are plotted as a function of the fulvic acid concentrations in sample filtrates at 1.5 h, as measured spectrophotometrically at 300 nm. Data for pyruvate and acetaldehyde fit well ($r^2 = 0.996$ and 0.997, respectively) to model curves computed from equation (1) using V_{max} and K values listed in the text. Because of scatter (presumably due to random contamination), formaldehyde rate data were not modelled. Acetone formation rates could not be measured because of insufficient analytical sensitivity.

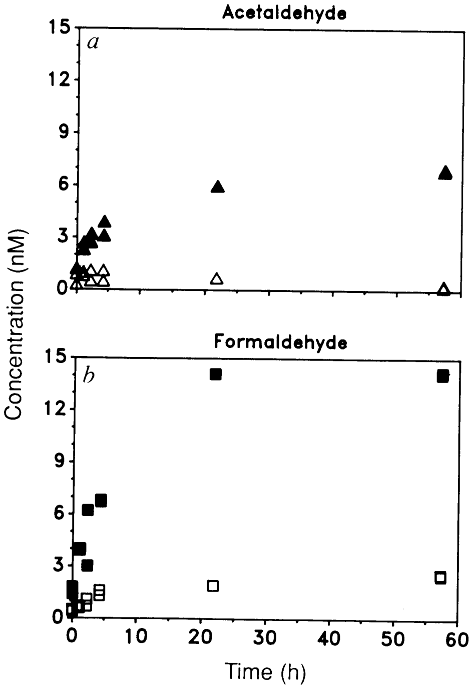

FIG. 3 Time course for formation of dissolved formaldehyde and acetaldehyde at pH 7.3 in Shark River estuarine water with (closed symbols) and without (open symbols) 1 mM added Mn oxides. α-Ketoacids were not measured.

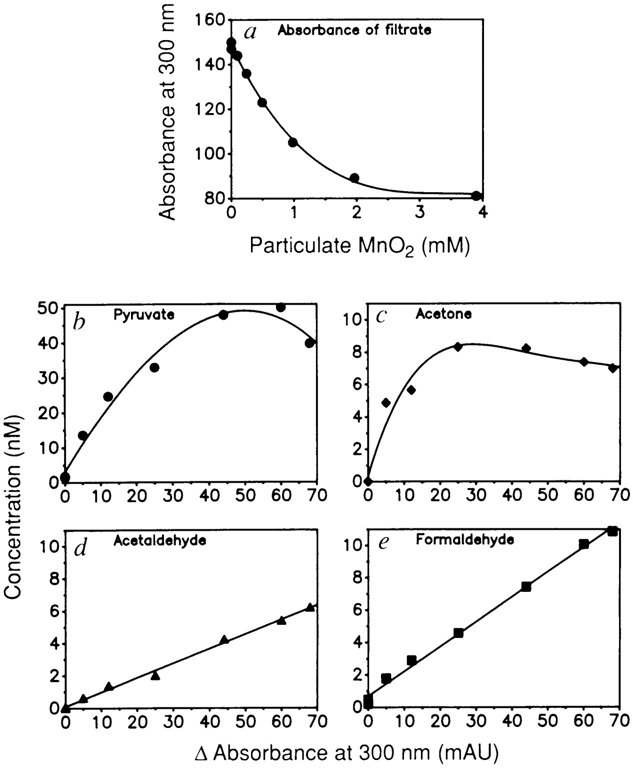

FIG. 4 Effect of added Mn oxides on loss of NOM absorbance (*a*) and net production of pyruvate (*b*), acetone (*c*), acetaldehyde (*d*) and formaldehyde (*e*) after 14.3 h reaction in Shark River estuarine water at pH 7.7 ± 0.1. Carbonyl values were corrected for blank values measured immediately after oxide addition and are plotted as functions of the loss of NOM absorbance at 300 nm for each Mn oxide addition (see *a*). Linear regression lines are drawn for formaldehyde and acetaldehyde data.

ate, acetone, acetaldehyde, and formaldehyde, respectively. The estimated yield for all four products was 0.46%. This value probably underestimates the overall yield for these compounds because some organic matter that adsorbs to the oxides may not react, and some stable products may not desorb following formation. Also, our calculations almost certainly underestimate the overall formation of LMW organic products, because our analytical methods would not detect several major classes of likely products such as organic acids (other than α-ketoacids) and alcohols.

The dissolved NOC in most oxidizing environments consists primarily of biologically refractory compounds such as humic and fulvic acids. This material is composed of complex mixtures of moderately high-molecular-weight organics whose structural diversity defies both specific molecular identification and coherent enzymatic attack. Thus, the oxidative lysis of these compounds by Mn oxides represents a mechanism by which biologically refractory organic matter is converted into a suite of low-molecular-weight compounds that can be used as microbial substrates. Pyruvate, the major LMW product that we identified, is a major metabolic intermediary in the cell's TCA cycle, and as such, provides a ready source of both energy and carbon for growth. It is also a major product of the photolysis of humic compounds by ultraviolet light, and its photochemical production is closely coupled to its microbial use in sea water[19].

In using LMW products, such as pyruvate, formed from photolysis of NOM[19], microorganisms can be viewed as exploiting an external photochemical process that is otherwise beyond their control. But this is not true in the case of microbial use of the same or similar compounds formed from Mn oxide lysis of NOM because Mn oxides in most natural environments are formed by microbial catalysis[3-6] and are found associated with the extracellular polymer sheath (glycocalyx) surrounding the bacterial cells[1,2]. Although several hypotheses have been proposed[1], the primary reason (or reasons) why the bacteria oxidize Mn (II) and deposit Mn oxides on their surfaces remains uncertain. We speculate that it may represent a means by which they can use the carbon contained in the large but biologically refractory pools of NOM, such as humic and fulvic acids. By mediating the production of Mn oxides these microbes also could play an important role in the biogeochemical cycling of organic carbon, particularly in surficial sediments where Mn oxide concentrations occur at much greater levels than those used in our experiments[6,10]. ☐

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